SUPPLEMENTARY INFORMATION



Supplementary Figure 1 - Functional characterization of DSGCs. **a**) Somatic calcium transients in 4 DSGCs with different PDs in response to bar stimuli moving in 8 different directions and **b**) Polar tuning curves for the 4 DSGCs color-coded by PD. Black lines indicate the direction of the vector-summed responses.



Supplementary Figure 2 - Skeleton reconstructions of SACs. a) Parallel (top) and normal (bottom) projections of 24 SACs (11 On SACs, 13 Off SACs, black). Scale bar is 100 μ m.



Supplementary Figure 3 - Specificity of On SAC output. **a**) an On SAC (black skeleton), with varicosities indicated by black dots. DSGC dendritic trees indicated by color-coded dashed ellipses. Synapses are color-coded by the PD of the post-synaptic DSGC. Scale bar is 50 µm.



Supplementary Figure 4 - Conventionally stained dataset. **a**) Block view, **b**) Partial skeleton of a bistratified GC (gray), presumably a DSGC (side view: left panel, normal view: right panel). Partial skeletons of presumed On SACs (11 skeleton fragments, cyan) and Off SACs (6 skeletons fragments, blue). **c**) Small cyan and blue dots indicate synapse locations (n=43); larger cyan and blue dots indicate the locations where the dendrite fragments exit the data set or reach the SAC soma. Only synapses where the distal end of a SAC dendrite was inside the data set or where the direction of the dendrite was obvious from the branching angles were used. **d**) Distribution of dendrite angles. Note similarity to Figure 5.



Supplementary Figure 5 - Directionality of SAC inputs to DSGCs. **a**) DSGC (grey skeleton), Connected On and Off SAC somata (large cyan and blue circles), and associated SAC/DSGC synapses (smaller cyan and blue circles). The vectors (cyan and blue lines) oriented from SAC somata to synapses are used to measure the dendrite angles. The distribution of the dendrite angles (black, lower right panel) and the functionally recorded tuning curve of this cell (red line) are shown. **b,c,d,e**) Same as a), but analyzed for 4 further DSGCs with 2 additional cardinal PDs (green, orange lines in polar plots). **f**) The soma locations for all traced DSGC and SAC somata in the dataset.

а



Supplementary Figure 6 - Definition of dendrite $(\theta_{dendr} - \theta_{ND})$ and soma $(\theta_{soma} - \theta_{ND})$ angles relative to the null direction of the DSGC and their difference $\gamma = (\theta_{soma} - \theta_{dendr}) \times \text{sign}(\theta_{soma} - \theta_{ND})$. Note that for this example the SAC soma is on the preferred side of the DSGC, but the synapse is oriented toward the null direction.

SAC 3

SAC 9

Supp Figure 7: Off SACs









SAC 18













SAC 22







Supplementary Figure 7 - Starburst amacrine cell gallery. The collection of Off and On SAC skeletons used in this study. Synapse locations are marked and color-coded as in Figure 4.